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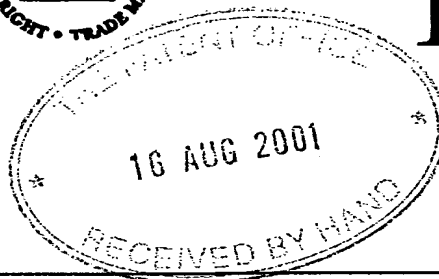
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1. Your reference SJK/BP5940754

2. Patent application number 16 AUG 2001 0120030.2
(The Patent Office will fill in this part)

3. Full name, address and postcode of the or of each applicant (underline all surnames) Medical Research Council 17AUG01 E653461-1 D00060
Patents ADP number (if you know it) 20 Park Crescent P01/7700 0.00-0120030.2
London W1N 1AL

If the applicant is a corporate body, give the country/state of its incorporation

00596007001
UK

4. Title of the invention Chitin Microparticles and Their Medical Uses

5. Name of your agent (if you have one) MEWBURN ELLIS

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

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Patents ADP number (if you know it)

109006

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number	Country	Priority application number (if you know it)	Date of filing (day / month / year)
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7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application	Date of filing (day / month / year)
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Description 15 ✓

Claim(s) 0

Abstract 0

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Priority documents 0

Translations of priority documents No

Statement of inventorship and right to grant of a patent (*Patents Form 7/77*) No

Request for preliminary examination and search (*Patents Form 9/77*) No

Request for substantive examination (*Patents Form 10/77*) No

Any other documents No
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11. I/We request the grant of a patent on the basis of this application.

Signature

Mauburn Ellis

Date

15 August 2001

12. Name and daytime telephone number of person to contact in the United Kingdom Simon J Kiddle 0117 926 6411

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Chitin Microparticles and Their Medical Uses

Field of the Invention

The present invention relates to chitin microparticles
 5 and their medical uses, in particular in the treatment of
 allergy, conditions that would benefit from an up-
 regulation of the cell mediated immune system and
 conditions that would benefit from an up-regulation of
 natural killer cell activity and/or the secretion of IFN-
 10 γ .

Background of the Invention

The alveolar macrophage is the most abundant leucocyte in
 the lumen of the alveolus and is central to the innate
 15 immune system of the lung by promoting phagocytic
 clearance and by the secretion of cytokines that promote
 an effective cell mediated immune response to inhaled
 particulates including microbes and pathogens. The
 principle cytokines produced during phagocytosis are IL-
 20 12, $\text{TNF}\alpha$, and IL-18. These macrophage cytokines
 subsequently induce IFN γ production by NK cells and Th1
 lymphocytes. IFN γ acts synergistically with these
 cytokines to promote a Th1 cell mediated immune response
 and also down-regulate the production of Th2 cytokines
 25 and in particular IL-4 and IL-5 which are strong
 mediators of allergy.

Studies by Shibata et al (2-5), have shown that oral
 delivery of 1-10 μm phagocytosable chitin particles
 30 results in an elevation of these Th1 cytokines in mouse
 spleen cell cultures. The effect was specific to the
 particulates as no elevation was produced by soluble
 chitin. It could also be reproduced in 1 μm polystyrene

microspheres coated with *N*-Acetyl-D-Glucosamine, which is the main component of chitin. It was also demonstrated that oral administration of chitin down-regulates serum IgE and lung eosinophilia in a murine model of ragweed allergy (2).

Shibata et al have also developed a mouse model of allergic airway inflammation and orally administered chitin preparations to the mice (Shibata 2000). Ragweed-specific IgE levels were significantly reduced after daily oral administration of chitin to ragweed-sensitised mice, before and during immunisation. Bronchioalveolar lavage (BAL) cells were harvested 14 days after immunisation and a reduction in the levels of eosinophil and lymphocyte levels was observed after chitin treatment. Lung inflammation was determined histologically 14 days after immunisation and the peribronchial, perivascular and total lung inflammation were inhibited in the chitin-treated group.

When chitin was administered prophylactically to mice who were subsequently administered ragweed, IL-4, IL-5 and IL-10 production was significantly reduced and low but significant levels of IFN- γ were detected.

Chitin also has a prophylactic effect when administered to C57BL6 mice, which are higher responders for cell-mediated immunity/Th1 responses, but lower responders for allergic responses compared with BALB/c mice.

When ragweed-sensitised mice were treated simultaneously with ragweed and chitin, the levels of IL-4, IL-5 and IL-10 produced were significantly reduced compared to those

stimulated by ragweed alone.

However, while Shibata et al disclose the use of chitin microparticles for the treatment of allergy, the compositions are administered orally as a supplement to activate macrophages and prophylactically strengthen the immune system in the absence of recurrent bacterial infections that are decreasingly common in industrialised countries.

More generally, existing treatments for allergies typically involve the use of steroids to depress the immune system. There are undesirable side effects with steroid therapy. Synthetic drugs, such as steroids are expensive to manufacture, involving a complex process which requires complex quality control and GMP standards to meet requirements of Health and Safety Authorities. In view of these factors, it remains a problem in the art in finding effective treatments for allergy.

Summary of the Invention

Broadly, the present invention relates to the use of chitin microparticle (CMP) preparations for treating disorders by delivering the microparticles intranasally to the sinuses and upper respiratory tract, e.g. using an intranasal spray, or by inhalation, e.g. targeting alveolar macrophages in the lungs.

The macrophage has a central control function in the innate immune system of the lung by promoting phagocytic clearance of particles and by processing the presentation of inhaled allergens to lymphocytes and by secretion of cytokines that promote an effective cell mediated immune

response to inhaled particulates including microbes and allergenic substance. In particular, the present invention discloses that intranasal delivery of chitin microparticles is particularly effective in reducing a number of parameters indicative of inflammation, thus providing as alternative to steroid treatments.

The work disclosed herein arises from the finding that the intranasal delivery of chitin microparticles to a mouse model of allergy produced by *Aspergillus fumigatus* (Afu) is particularly effective in reducing levels of peripheral blood eosinophilia, serum total IgE and Afu-specific IgG1.

Accordingly, in a first aspect, the present invention provides the use of a chitin microparticle (CMP) preparation for the preparation of a medicament for treating allergy, wherein the medicament is delivered intranasally or by inhalation.

In an alternative aspect, the present invention provides a method of treating a patient suffering from allergy, the method comprising administering to the patient a therapeutically effective amount of a chitin microparticle preparation, wherein the CMP preparation is administered intranasally or by inhalation.

Examples of allergies that can be treated according to the present invention include seasonal respiratory allergies, commonly referred to as hay fever; allergy to aeroallergens including house mite dust, fungal spores, grass pollens, tree pollens and animal danders; allergy treatable by reducing serum IgE and eosinophilia; asthma;

eczema and food allergies.

5 In a further aspect, the present invention provides the use of a chitin microparticle (CMP) preparation for the preparation of a medicament for the treatment of conditions that would benefit from the up-regulation of the cell-mediated immune system, wherein the medicament is administered intranasally or by inhalation.

10 In an alternative aspect, the present invention provides a method of treating a patient suffering from a condition that would benefit from the up-regulation of the cell-mediated immune system, the method comprising administering to the patient a therapeutically effective
15 amount of a chitin microparticle preparation, wherein the CMP preparation is administered intranasally or by inhalation.

Examples of such conditions include the treatment
20 microbial infections, lung infections; pulmonary viral infections such as respiratory syncytial virus bronchiolitis, especially in infants and the elderly, or influenza virus, or rhino virus; fungal infections such as invasive pulmonary aspergillosis and invasive
25 pulmonary candidiasis, e.g. in immunosuppressed patients; and bacterial pneumonias.

In a further aspect, the present invention provides the use of a chitin microparticle (CMP) preparation for the
30 preparation of a medicament for the treatment of conditions treatable by up-regulation of the activity of Natural Killer cells and/or secretion of interferon- γ by cells of the immune system, wherein the medicament is

administered intranasally or by inhalation.

In an alternative aspect, the present invention provides a method of treating a patient suffering from a condition
5 treatable by up-regulation of the activity of Natural Killer cells and/or secretion of interferon- γ by cells of the immune system, the method comprising administering to the patient a therapeutically effective amount of a chitin microparticle preparation, wherein the CMP
10 preparation is administered intranasally or by inhalation.

An example of a condition treatable in this aspect of the invention is lung cancer.

15 Preferably, the medicaments set out above are for administration to humans. Preferred patient groups for intranasal treatment with CMP would include those suffering from seasonal rhinitis and sinusitis, or
20 chronic respiratory allergies such as house dust mite allergy and who are currently taking steroids or antihistamines. Other groups include hospitalised patients being treated for chronic lung disorders including infections and lung carcinomas.

25 Chitin is a polymer of N-acetyl-D-glucosamine and has a similar structure to cellulose. It is an abundant polysaccharide in nature, comprising the horny substance in the exoskeletons of crab, shrimp, and insects as well
30 as fungi. Any of these or other sources of chitin are suitable for the preparation of CMP preparations for use according to the present invention.

Preferably, chitin is produced by physically reducing it, e.g. by sonication, to particles having a diameter of less than 50 μm , more preferably less than 40 μm , still more preferably less than 20 μm , more preferably less than 10 μm and most preferably less than 5 μm . An upper limit of chitin particles size is functionally defined by macrophages not recognising the particles. As a lower limit, preferably the particles are at least 1 μm in diameter. The lower size limit is functionally defined by the chitin particles becoming soluble and hence also not being recognised by macrophages. Size can readily be determined by the skilled person for example using flow cytometry or a microscope. Alternatively or additionally, the chitin microparticles can be made by coating a carrier particles, e.g. formed from a biocompatible material such as polystyrene or latex, with *N*-Acetyl-D-Glucosamine, chitin or a fragment thereof, to form particles having the sizes as defined above.

Within a population of chitin microparticles forming a CMP preparation, preferably at least 90%, and more preferably 95% and most preferably at least 99%, of the chitin particles have a size distribution within the limits set out above.

In a further aspect, the present invention provides an delivery device comprising a reservoir of chitin microparticles as defined herein, and a delivery orifice adapted to locate in a patient's mouth or nose, wherein the patient can place the delivery orifice in the mouth or nose to administer the chitin microparticles. In some embodiments the device may comprise a valve between the reservoir and the delivery orifice, such that the valve

can be operated to control delivery of the chitin microparticles. The microparticles may be drawn into the nose to the sinuses and upper respiratory tract or through the mouth to the alveolar macrophages by inhalation and/or by a propellant. A particularly preferred form of device is a nasal spray bottle containing a CMP preparation and optionally a carrier, the spray bottle having a neck adapted for nasal delivery.

In addition to chitin microparticles, the CMP preparations can comprise one or more of a pharmaceutically acceptable excipient, carrier, propellant, buffer, stabiliser, isotonicizing agent, preservative or anti-oxidant or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient.

Preservatives are generally included in pharmaceutical compositions to retard microbial growth, extending the shelf life of the compositions and allowing multiple use packaging. Examples of preservatives include phenol, meta-cresol, benzyl alcohol, para-hydroxybenzoic acid and its esters, methyl paraben, propyl paraben, benzalconium chloride and benzethonium chloride. Preservatives are typically employed in the range of about 0.1 to 1.0 % (w/v).

Preferably, the pharmaceutically compositions are given to an individual in a "prophylactically effective amount" or a "therapeutically effective amount" (as the case may be, although prophylaxis may be considered therapy), this

being sufficient to show benefit to the individual, e.g. providing alleviation of allergy or prophylaxis for an acceptable period. Typically, this will be to cause a therapeutically useful activity providing benefit to the individual. The actual amount of the compounds administered, and rate and time-course of administration, will depend on the nature and severity of the condition being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 16th edition, Osol, A. (ed), 1980. By way of example, and the compositions are preferably administered in dosages of between about 0.01 and 100mg of active compound per kg of body weight, and more preferably between about 0.5 and 10mg/kg of body weight. By way of example, this could be achieved using a nasal delivery bottle to deliver 4-8 doses of approximately 0.25ml of a 5 mg/ml solution of CMP particles.

Embodiments of the present invention will now be described by way of example and not limitation with reference to the accompanying figures.

30 Brief Description of the Figures

Figure 1 shows the results of treatment with 17 μ g chitin microparticles (CMP) which produced a significant decrease ($p < 0.05$) in peripheral blood eosinophilia.

Figure 2 shows a reduction in serum total IgE ($p < 0.0005$) after treatment with 17 μ g/day of CMP microparticles.

5 Figure 3 shows a reduction in Afu specific IgG1 ($p < 0.01$).

Detailed Description

Materials and Methods

Chitin microparticles delivered intranasally represent a
10 new approach to stimulating cell mediated immunity and promoting anti-inflammatory responses in inflamed tissues. The present invention has the considerable advantage that macrophages of the upper respiratory tract or alveolar macrophages can be directly targeted with
15 chitin microparticles of the correct size using an intranasal spray and inhalation delivery respectively.

A mouse model has been established to demonstrate the efficiency of the present invention.

20

The parameters measured in the present study are serum IgE and IgG1 and peripheral blood eosinophilia, which are all significantly elevated in the mouse model of allergy to *Aspergillus fumigatus* allergens and all significantly
25 reduced by intranasal treatment with CMP. It is proposed that the mode of action is that the CMP are bound by the mannose receptors of macrophages in the nasal mucosa and alveolae, which stimulates them to generate IL-12, TNF- α and IL-18, which lead to the generation of IFN- γ by NK
30 cells and Th1 lymphocytes. All these cytokines and particularly IFN- γ , promote a shift in the populations of T lymphocytes from Th2 to Th1. This culminates in the observed reduction in serum IgE and eosinophilia, which

are major components in allergy.

Chitin Microparticle Suspension Preparation (CMP)

Chitin microparticles were prepared from purified chitin
5 (Sigma Chemical Co., St. Louis, MO) by sonication of a
suspension of 10 mg/ml in endotoxin free PBS at maximum
output for 20 min. with cooling on ice every 5 min. The
slurry was centrifuged at 1000xg for 10 min to remove
large particles and the microparticles were collected by
10 centrifugation at 4000xg and washed 3 times with PBS to
remove any solubilized chitin. The supernatant contained
a uniform suspension of small particles as judged by
light microscopy using a haemocytometer with 50 μm
squares and were comparable in size to 1 μm latex spheres
15 (Polysciences, Inc.). Particles less than 5 μm in
diameter were quantified with a Celltac Hematology
Analyser (Nihon Kohden, Inc.). Preparations were found
to contain 99.9% microparticles less than 5 μm in
diameter and at a concentration in the order of 10^{11} /ml.

20

Endotoxin was measured by Limulus Amebocyte Lysate Assay
(BioWhittaker Co,) and shown to be <1 EU/ml.

Aspergillus fumigatus antigen (Afu 1wcf)

25 *Aspergillus fumigatus* (Afu) was grown in a synthetic
medium (M199, Sigma Chemicals) as a stationary culture
for 1 week at 37°C. Arrunda et al, demonstrated that the
expression of Asp f1, a major allergen, is maximal after
1 week and tends to diminish during longer incubation
30 periods (Arrunda 1992). The 1 week culture was killed by
adding 0.1% Thimerosal for 12 hours. The culture was
filtered through glass wool and finally through a 0.45 μm
membrane to remove all particulates and possible spores

and then dialysed with 3 buffer changes against water.
The dialysate was lyophilised to give a brown powder.

5 A major band at 18kDa corresponds to Asp f 1. A band
corresponding to Asp f 2 (37kDa) is also evident. The
18kDa band was N-terminal sequenced giving the sequence
ATWTCINQQLNP, corresponding to the N-terminal sequence
for Asp f 1.

10 It was also demonstrated by ELISA that the 1-week culture
filtrate (1wcf) was recognised by human serum from Afu-
allergic patients obtained from the National Institute of
Biological Standards and Control.

15 Sensitisation

Mice (Female C57Bl/6, 6 weeks old) were sensitised by
intra-peritoneal (i.p.) injections of Afu 1wcf with
alum (1:3 antigen : alum) over 3 weeks followed by
intranasal instillation (i.n.) with 5 daily doses of 50
20 μ l containing 10 μ g of Afu 1wc supplemented with PBS or
CMP.

Peripheral blood eosinophils

Blood was collected from the tail vein of the mice (n=4-
25 5/group) for estimation of eosinophils. Total leukocyte
count was determined by an automatic cell counter and the
% eosinophils was determined by differential counting by
microscope of slides of stained blood smears. Results
are expressed as 10^6 cells / ml.

30

Serum IgE and Afu-IgG1

Total serum IgE was measured by sand-which ELISA
(PharMingen kit) in blood serially diluted from a maximum

dilution of 1:20 to give values which were linear with respect to a standard curve of mouse IgE. Results are expressed in $\mu\text{g/ml}$. Afu-specific IgG1 was measured by ELISA with ELISA plates coated with Afu 1wcf. Antibody
5 was detected with HRP-labelled anti-mouse IgG1. Results are expressed as absorbance units (OD450).

Results

Example 1

10 The effect of treatment with CMP on blood eosinophilia is shown in Figure 1. Groups had received treatment for 4 days and measurements were made on day 5. The sample size was 4-5 mice/group. Error bars \pm SEM. The results indicate that treatment with CMP resulted in a drop in
15 the blood eosinophilia level to $\text{ca } 0.3 \times 10^6/\text{ml}$, compared with test animals treated with PBS which exhibited blood eosinophilia levels of $\text{ca } 0.7 \times 10^6/\text{ml}$.

Example 2

20 A comparison of the effect of treatment with CMP on serum IgE levels of mouse models is shown in Figure 2. Groups were treated for 5 days and measurements were made on blood collected 3 days later. The sample size was 4-5 mice/group. Error bars \pm SEM. The results indicate that
25 Serum IgE levels 3 days after treatment with CMP are less than 5 $\mu\text{g/ml}$ IgE, compared with 24 $\mu\text{g/ml}$ IgE in test animals that had not received intranasal treatment.

Example 3

30 A comparison of the effect of treatment with CMP or rSP-D on serum IgG1 levels is shown in Figure 3. Groups were treated for 5 days and measurements were made on blood collected 3 days later. The sample size was 4-5

mice/group. Error bars \pm SEM. Test animals infected with Afu 1wcf and subsequently treated with CMP show a four fold decrease in serum IgG1 levels compared with infected animals who did not receive CMP.

5

References

The references mentioned herein are all expressly incorporated by reference.

- 5 1. Arruda et al, J. Immunol. 149:3354-9, 1992.
2. Shibata et al, J. Immunol., 164: 1314-1321, 2000.
3. Shibata et al, J. Immunol., 161: 4283-8, 1998.
- 10 4. Shibata et al, Infection and Immunity, 65(5): 1734-1741, 1997.
5. Shibata et al, J. Immunol., 159: 2462-2467, 1997.

15

Claims

1. Use of a chitin microparticle preparation for the preparation of a medicament for the treatment of allergy, wherein the medicament is delivered intranasally or by inhalation.

2. The use of claim 1, wherein the allergy is seasonal respiratory allergy, allergy to aeroallergens including house dust mite, fungal spores, grass pollens, tree pollens and animal danders; allergy treatable by reducing serum IgE and eosinophilia; asthma; eczema or a food allergy.

3. Use of a chitin microparticle preparation for the preparation of a medicament for the treatment of a condition that would benefit from the up-regulation of the cell mediated immune system, wherein the medicament is administered intranasally or by inhalation.

4. The use of claim 3, wherein the condition comprises microbial infections, lung infections; pulmonary viral infections such as respiratory syncytial virus bronchiolitis, influenza virus, or rhino virus; fungal infections such as invasive pulmonary aspergillosis and invasive pulmonary candidiasis or bacterial pneumonias.

5. Use of a chitin microparticle preparation for the preparation of a medicament for the treatment of a condition by up-regulation of the activity of NK cells and/or secretion of IFN- γ by cells of the immune system, wherein the medicament is administered intranasally or by inhalation.

6. The use of claim 5, wherein the condition is lung cancer.

5 7. The use of any one of the preceding claims, wherein at least 90% of the chitin microparticles have a diameter within the range of 1-5 μ m.

10 8. The use of any one of the preceding claims, wherein the chitin microparticles are derived from the exoskeletons of crab, shrimp, lobster, and insects and fungi.

15 9. The use of any one of the preceding claims wherein the medicament is administered to a patient in a therapeutically effective amount of between 0.01 and 100mg of active compound per kg of body weight.

20 10. The use of any one of the preceding claims wherein the medicament is administered to humans.

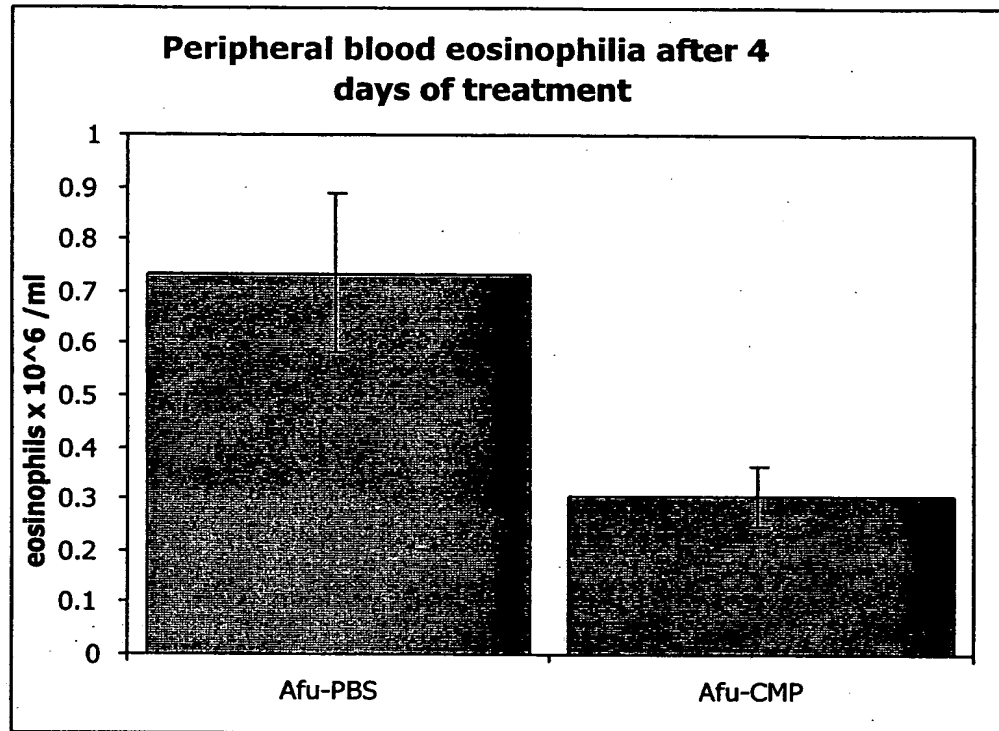
25 11. The use of any one of the preceding claims wherein the chitin microparticle preparation comprises one or more of a pharmaceutically acceptable excipient, a carrier, a propellant, a buffer, a stabiliser, an isotonicizing agent, a preservative or an antioxidant.

30 12. A delivery device for the administration of the chitin microparticles of any one of the preceding claims, comprising:

- a) a reservoir of chitin microparticles;
- b) a delivery orifice adapted to locate in a patient's mouth or nose; and
- c) a valve between the reservoir and the delivery

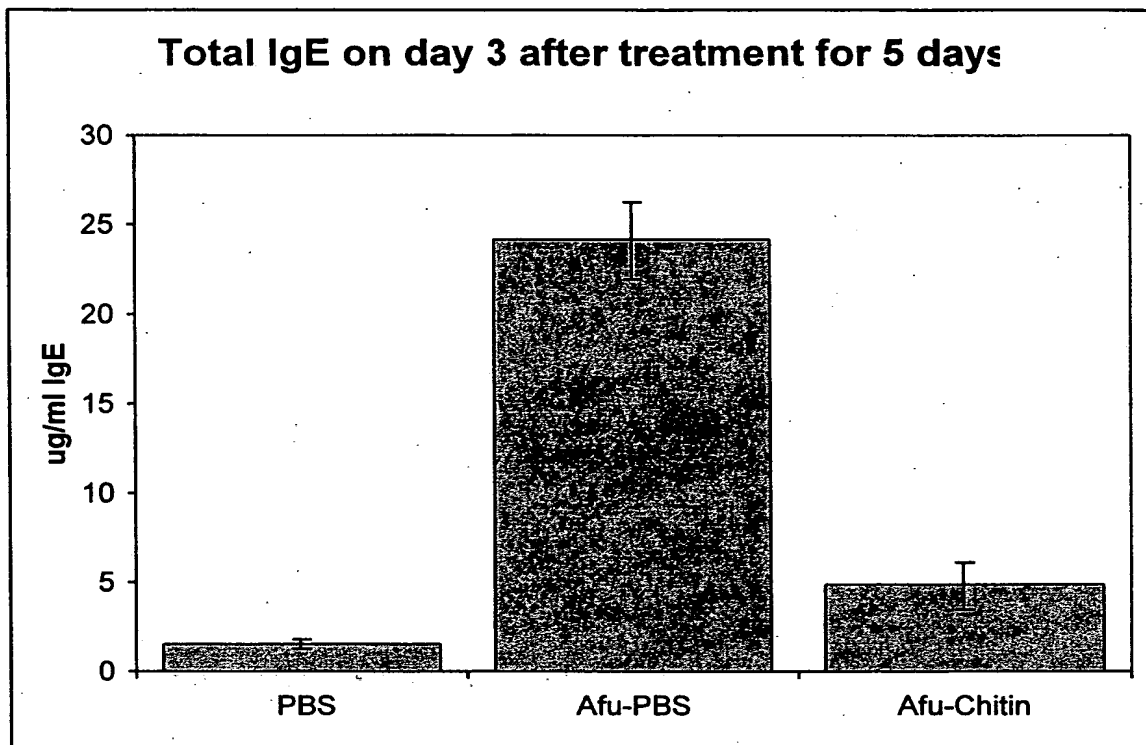
orifice such that the valve can be operated to control delivery of the chitin microparticles.

Figure 1



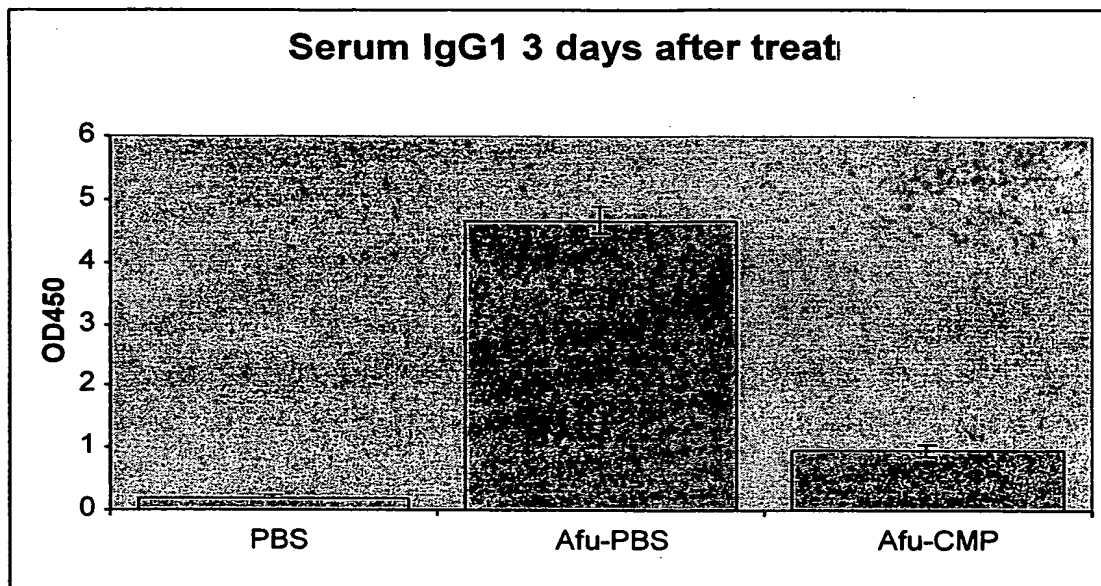
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Figure 2



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Figure 3



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